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Rapid, non-invasive measurement of gastric emptying rate using transcutaneous fluorescence spectroscopy

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Abstract: Gastric emptying rate (GER) signifies the rate at which the stomach empties following ingestion of a meal and is relevant to a wide range of clinical conditions. GER also represents a rate limiting step in small intestinal absorption and so is widely assessed for research purposes. Despite the clinical and physiological importance of gastric emptying, methods used to measure GER possess a series of limitations (including being invasive, slow or unsuitable for certain patient populations). Here, we present a new technique based on transcutaneous (through-the-skin) fluorescence spectroscopy that is fast, non-invasive, and does not require the collection of samples or laboratory-based analysis. Thus, this approach has the potential to allow immediate reporting of clinical results. Using this new method, participants receive an oral dose of a fluorescent contrast agent and a wearable probe detects the uptake of the agent from the gut into the blood stream. Analysis of the resulting data then permits the calculation of GER. We compared our spectroscopic technique to the paracetamol absorption test (a clinically approved GER test) in a clinical study of 20 participants. Results demonstrated good agreement between the two approaches and, hence, the clear potential of transcutaneous fluorescence spectroscopy for clinical assessment of GER.

1. Introduction

Gastric emptying rate (GER) represents the rate at which the stomach empties following ingestion of a meal and is reflective of a variety of physiological and food-related functions. GER is therefore commonly measured in research into gastro-intestinal processes such as digestion and absorption, and it also has importance clinically [1]. In particular, delayed gastric emptying (gastroparesis) has been reported in wide-ranging conditions including functional dyspepsia [2–4], gastro-oesophageal reflux disease [5], diabetes [6–10] and even stomach cancer [11].

Several methods to measure GER are in clinical use, most of which entail oral ingestion of a contrast agent or marker (usually contained within a test meal) followed by imaging of gastric emptying or detection of the marker/agent in the blood or breath [12]. Techniques include direct imaging methods such as gastric scintigraphy and indirect tracer methods such as stable isotope breath tests and the paracetamol absorption test [12, 13].

Scintigraphy is regarded as the clinical gold standard. However, it involves radiation exposure, lacks standardisation with regards to meal composition, patient positioning and timing of image acquisition, is expensive, and requires nuclear medicine facilities [14, 15]. These drawbacks render it unfavourable, nonviable or unavailable in certain patient groups and populations.

Stable isotope breath tests, which provide an indirect assessment of GER, are simple to administer and minimally invasive [16–18]. However, they require specialised equipment and facilities to produce and analyse tests. In addition, they can be expensive and there is a time delay between administering the test and reporting results due to the need for laboratory-based analysis of the collected breath samples. Breath tests can also be inaccurate or not possible in patients with diseases affecting the intestinal mucosa, pancreas, liver or respiratory system.

The paracetamol absorption test is another approach that provides indirect assessment of gastric emptying [14, 15, 19]. The test is both economical and simple to perform, and it works as orally administrated paracetamol is poorly absorbed by the stomach but rapidly absorbed in the small intestine. Thus, as gastric emptying is the rate limiting step in delivering paracetamol to the absorption site, the rate of appearance of paracetamol in the blood indirectly reflects GER. Nonetheless, the method requires collection of numerous (typically 10-20) blood samples as well as facilities for sample collection and analysis. Furthermore, like stable isotope breath tests, there is a time delay between test and results due to the laboratory-based measurement of paracetamol concentrations in the blood samples.

Finally, all of the above methods require samples/images to be collected over extended time periods (typically 3-4 hours) to ensure that the full gastric emptying process is captured. As a result, there is a clear need for rapid and non-invasive alternative tests for GER to facilitate clinical uptake on a larger scale.

We have recently demonstrated the potential of transcutaneous fluorescence spectroscopy as a tool for non-invasive monitoring of gut function [20]. This technique – which is analogous to experiments previously undertaken in animals (e.g. [21–24]) – involves patients receiving an oral dose of a fluorescent contrast agent and a wearable fibre-optic probe being used to monitor the uptake of those agents from the gut into the blood stream. Measurements are performed through the skin and are thus almost entirely non-invasive. Furthermore, by assessing parameters such as the rate of uptake and/or intensity of the fluorescence signal, it may be possible to quantify important clinical factors such as intestinal permeability or GER [20].

Here we report results from a first-in-human trial examining the use of this novel approach for assessment of GER in healthy volunteers. Fluorescence data are compared against paracetamol absorption tests revealing that the fluorescence-based approach permits rapid, non-invasive measurement of GER with the potential to report clinical results in hours rather than days. This approach is almost completely non-invasive and does not require collection of urine, blood or even breath samples (as necessitated by other clinical GER tests). In addition, it is considerably cheaper and faster than gastric scintigraphy, the gold standard method for quantification of GER. Moreover, this spectroscopic method uses fluorescein as a contrast agent, which is already approved for use in other clinical procedures. This indicates potential for rapid translation into widespread clinical use both for assessment of GER and for other conditions/applications.

2. Materials and methods

2.1. Portable fibre-optic fluorescence spectrometer

In the experiments presented here, a fibre-optic spectrometer was used to assess fluorescence signals at the fingertip. This system is described in detail in [20] and a schematic diagram of the optical setup is shown in Fig. 1(A). Briefly, the system comprises two laser sources (wavelengths of 488 nm and 785 nm) for excitation of fluorescence (Stradus 488-25 and Stradus 785-80, Vortran Laser Technology, USA), which are coupled into a bifurcated fibre-optic probe (QR200-7-VIS-NIR, Ocean Optics, The Netherlands) to allow interrogation of fluorescence signals at the skin. At the laser outputs, bandpass and neutral density (ND) filters are used to clean up the emission profiles and to limit the optical power to safe levels. The fluorescence signal is collected by the detection channel of the fibre probe, the output of which is then directed through an emission filter wheel (which contains long pass filters to reject any scattered laser

light) and into a multimode optical fibre (P600-1-VIS-NIR, Ocean Optics, The Netherlands) that routes the light to a spectrometer for detection (FLAME-S-VIS-NIR-ES, Ocean Optics, The Netherlands). In the experiments presented here only the 488 nm laser was used, as this provided efficient excitation of fluorescence from fluorescein.
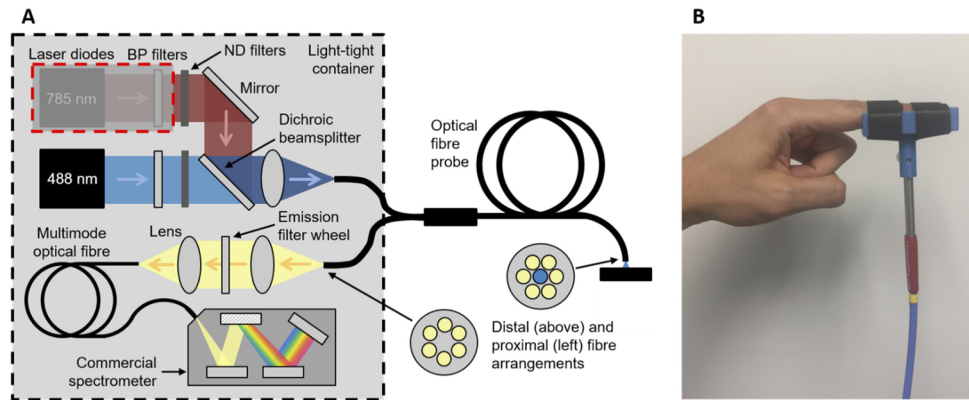

Fig. 1. Portable fibre-optic fluorescence spectrometer used for transcutaneous detection of fluorescence signals following oral ingestion of fluorescein (as part of a liquid test meal). **(A)** Schematic diagram of the optical system. Insets show the arrangements of optical fibres in the bifurcated fibre probe (excitation fibre – blue; collection fibres – yellow). Dotted red box around the 785 nm laser indicates that this light source was not used in this study. **(B)** Photograph of the 3D printed wearable mount that was used to secure the tip of the optical fibre probe in contact with the fingertip. Figure amended from Ref. [20] under the terms of the Creative Commons CC BY license.

The optical power (at 488 nm) at the fibre probe output was limited to a maximum of 63 µW (using both the ND filters and the laser control software – Stradus GUI Software, Vortran Laser Technology, USA). This ensured that the emitted radiation was below the maximum permissible exposure for skin and that the system was eye-safe as long as the probe was held more than 10 cm from the eyes at all times [25–27].

The fibre-optic spectrometer was controlled using a laptop computer running custom-written LabVIEW software, which allowed automated data collection. Following ingestion of a liquid test meal containing fluorescein (see details in *Liquid test meal* section below), fluorescence spectra were recorded at the fingertip at one minute intervals for a total of 250 minutes. For each time point, the integration time was determined automatically by the software to ensure that a sufficient signal-to-noise ratio was obtained. This was achieved by recording and summing repeated spectra with 500 ms integration times until a chosen signal threshold was reached. At each time point, a spectrum of the backscattered laser signal was also recorded (1 ms integration time, 50 averages) by setting the emission filter wheel to an empty position. These laser spectra were used to normalise the fluorescence spectra (see *Calculation of normalised integrated fluorescence values* section below) to account for fluctuations due to factors including variations in laser power and movement of the fibre probe.

Finally, to perform transcutaneous fluorescence measurements, the fibre-optic probe was held in contact with the forefinger of the participants using a 3D-printed mount and two Velcro straps (see Fig. 1(B)). This mount acted to secure the tip of the fibre probe in gentle contact with the skin on the forefinger.

2.2. Clinical trial measurements

Clinical measurements were performed at St. Mary's Hospital, London, UK. A total of 20 healthy volunteers (with no active gastro-intestinal or liver disease) were recruited to the trial and all gave informed consent prior to inclusion in the study. All experiments were performed according to the clinical study protocol [28] (details of ethical approval: UK Health Research Authority IRAS Project ID – 242462; Research Ethics Committee reference – 18/LO/0714) and in accordance with Good Clinical Practice (GCP) guidelines and the World Medical Association's Declaration of Helsinki.

Following confirmation of eligibility, participants were asked to fast overnight prior to their assigned study day. On the morning of the trial, participants first gave informed consent and were then asked a series of questions about their diet and general gastro-intestinal health. Female participants were also asked to take a urine pregnancy test (Alere^{TM} hCG Easy, Abbot, USA) to ensure that pregnant women were not included in the study.

Before the experiments began, the fibre-optic probe was attached to the participant's forefinger and the participant was cannulated (below the elbow) to allow collection of venous blood samples for measurement of paracetamol concentrations. The first (baseline) blood sample was collected and then fluorescence measurements were started at the same time that the participant was asked to begin drinking the liquid test meal (which contained both fluorescein and paracetamol – see details below). Participants all consumed the entire liquid test meal within 3 minutes. Fluorescence spectra were recorded at one minute intervals for the remainder of the experiment. Blood samples were collected at 10 minute intervals for the first hour after ingestion of the liquid test meal and then at 15 minute intervals thereafter. Blood samples were 3 ml and were collected within serum-separated tubes. Following collection, blood samples were taken to Imperial College Trust Pathology (St Mary's Hospital) for analysis, where plasma paracetamol levels were quantified using an enzymatic colorimetric assay with the use of an Olympus AU600 Analyser. In total, the measurements lasted for 4 hours and 10 minutes, and the participant was seated for the duration of the experiments.

In the analysis of data, two datasets were excluded. The paracetamol data for participant 7 was excluded from the calculation of percentage retention (see below) as a steady state elimination phase was not reached (which is required for calculation of percentage retention using paracetamol absorption tests [19]). The fluorescence data for participant 9 was excluded from all analysis as a manual error in data collection meant that this dataset could not be correctly normalised (according to the procedure described below).

2.3. Liquid test meal

The liquid test meal was designed according to guidelines for standardisation of paracetamol absorption tests for gastric emptying assessment [14]. A 440 ml, 350 kcal liquid meal (representing a typical chocolate milkshake) was used. The milkshake was composed of approximately 28% Protein, 20% fat and 50% carbohydrate. Both tracers were added to the liquid test meal: 1.5 g paracetamol was dissolved in the liquid test meal, and 5 ml (500 mg) fluorescein was dispersed into the liquid test meal. The ingredients of the liquid test meal are shown in Table 1. The liquid test meal was served in a glass (with a straw) and participants were asked to consume the entire meal within 3 minutes of the beginning of the experiments. Fluorescein was purchased from Imperial College Healthcare NHS Trust Pharmacy (Anatera, Alcon, Novartis Pharmaceuticals, UK) and was provided in aqueous solution (500 mg dissolved in 5 ml water). Paracetamol was procured from Sigma-Aldrich in powdered form (Acetaminophen, A5000-100G, Sigma-Aldrich Ltd., UK).

Table 1. Ingredients of the liquid test meal (milkshake) used for assessment of GER using both transcutaneous fluorescence spectroscopy and the paracetamol absorption test.

Ingredient	Amount
Nesquik Chocolate powder	27 g
Semi-skimmed milk	400 ml
Nutricia PROTIFAR – High Protein Powder	10.4 g
Sugar	2.88 g
Fluorescein	500 mg / 5 ml
Paracetamol	1.5 g

2.4. Calculation of normalised integrated fluorescence values

To allow comparison of data between time points and between participants, we used the measured fluorescence and laser spectra to calculate a normalised, integrated fluorescence value for each time point in each acquisition. This analysis process is described in detail in Ref. [20]. Briefly, the fluorescence and laser spectra were first background subtracted, with the background value for each spectrum calculated as the average intensity over the wavelength range 350–450 nm (as no signal was observed in this region). The background-subtracted spectra were then summed (integrated) over the wavelength range containing the spectral peak (500–580 nm for the fluorescence spectra; 485–492 nm for the laser spectra). The resulting integrated fluorescence values were then normalised according to both laser power (by dividing by the corresponding integrated laser spectrum value) and acquisition time. This was achieved according to Eq. (1) below, where I_{int} is the integrated, normalised fluorescence intensity, λ is the wavelength, t_F and t_L are the integration times for the fluorescence and laser spectra respectively, $I(\lambda)$ is the fluorescence spectrum, $L(\lambda)$ is the laser spectrum, and B_F and B_L represent the background values for the fluorescence and laser spectra respectively [20].

$$I_{int} = \frac{\left(\sum_{\lambda=500nm}^{\lambda=580nm}(I(\lambda) - B_F)\right) \times t_L}{\left(\sum_{\lambda=485nm}^{\lambda=492nm}(L(\lambda) - B_L)\right) \times t_F} \tag{1}$$

2.5. Calculation of percentage retention values based on fitting of fluorescence vs. time curves

As discussed further in the *Results* section, percentage retention values (the percentage of the liquid test meal remaining within the stomach) were calculated from the fluorescence data in two ways: either directly (i.e. according to Eq. (8) – see *Results*) or by fitting a function to the data. In the fitting-based approach, the following function was fitted to the fluorescence vs. time data:

$$I(t) = (1 - Ct)(B(t) + L(t)). \tag{2}$$

In Eq. (2), $I(t)$ represents the fluorescence intensity as a function of time (t), C represents the rate at which fluorescein is eliminated from the body, $B(t)$ represents the fluorescence intensity contribution from fluorescein in the blood stream (as a function of time, t), and $L(t)$ represents the fluorescence intensity contribution from fluorescein that has leaked into the epithelium (as a function of time, t). The parameters $B(t)$ and $L(t)$ were defined as sigmoid functions, which represent the uptake of fluorescein from the gut into the blood stream ($B(t)$) and the leakage of fluorescein from blood vessels into the skin's epithelium ($L(t)$) respectively. Specifically, $B(t)$

and $L(t)$ were defined according to

$$B(t) = \frac{B_{max}}{1 + exp\left(-k_B\left(t - t_{B\frac{1}{2}}\right)\right)} \tag{3}$$

and

$$L(t) = \frac{L_{max}}{1 + exp\left(-k_L\left(t - t_{L\frac{1}{2}}\right)\right)} \tag{4}$$

In the above equations, B_{max} represents the maximum intensity contribution (to the fluorescence signal) from fluorescein in the bloodstream, and k_B and $t_{B\frac{1}{2}}$ respectively represent the logistic growth rate (steepness) of the $B(t)$ curve and the time at which $B(t)$ reaches half of its maximum value. Similarly, L_{max} represents the maximum intensity contribution from fluorescein in the epithelium, and k_L and $t_{L\frac{1}{2}}$ represent the logistic growth rate and half-maximum time point for the $L(t)$ curve.

Substituting Eqs. (3) and (4) into Eq. (2) gives the full form of the fitting function used in this analysis:

$$I(t) = (1 - Ct)\left(\frac{B_{max}}{1 + exp\left(-k_B\left(t - t_{B\frac{1}{2}}\right)\right)} + \frac{L_{max}}{1 + exp\left(-k_L\left(t - t_{L\frac{1}{2}}\right)\right)}\right) \tag{5}$$

Thus, for each fluorescence vs. time curve, Eq. (5) was fitted to the data in order to extract the parameters used to calculate both the amount of fluorescein that had emptied from the stomach at time t ($S(t)$) and the percentage retention (R). The calculations of $S(t)$ and R were performed according to Eqs. (6) and (7) respectively (see *Results*). Fitting was achieved using MATLAB code written in-house that performed least squares fitting using the 'lsqcurvefit' inherent MATLAB function.

2.6. Statistical analysis

Statistical tests were performed to compare the paracetamol and fluorescence datasets. First, we compared the times at which peak values were observed in the fluorescence and paracetamol vs. time curves. The paracetamol peak times were compared against the first and second peaks in the fluorescence data and against the average (mean) of the two fluorescence peak times. Second, we compared the times at which 75%, 50% and 25% retention were observed in the paracetamol and fluorescence percentage retention datasets. The paracetamol retention times were compared against the fluorescence retention times calculated using both the fitting-based analysis and the direct method of calculation. In all cases, Wilcoxon signed rank tests were used to make the statistical comparisons, and statistically significant differences were inferred for p-values of less than 0.05. To allow paired analysis, data for participant 9 was excluded from the comparison of peak times. Similarly, data for participants 7 and 9 were excluded from the comparison of retention times. The reasons for these exclusions are described above (see *Clinical trial measurements*).

3. Results

3.1. Comparison of transcutaneous fluorescence and paracetamol absorption time courses

To investigate the potential of transcutaneous fluorescence spectroscopy as a tool for non-invasive assessment of GER, 20 healthy volunteers were recruited to a clinical trial to take simultaneous paracetamol absorption and fluorescence-based GER tests. The paracetamol absorption test is a

clinically approved technique for assessment of GER and thus acted as a method against which the fluorescence-based approach could be validated.

A portable, fibre-optic spectrometer was used to record fluorescence profiles. This system – which was presented previously [20] and is described in detail in the *Materials and methods* – entails a laser source at 488 nm for excitation of fluorescence from fluorescein and a flexible fibre-optic probe to permit interrogation of fluorescence signals at the skin (see Fig. 1).

Following recruitment to the trial, participants were asked to fast overnight prior to inclusion in the study. On the day of their study visit, participants were cannulated to allow collection of blood samples for paracetamol absorption tests and the fibre-optic probe was attached to their index finger using a 3D printed mount for collection of fluorescence data (see Figs. 2(A)&B). After collection of a baseline blood sample, participants were asked to consume a liquid test meal (a milkshake) containing both paracetamol and fluorescein (see details in *Materials and methods*). Fluorescence data and blood samples were then collected for the following four hours. Following data/sample collection, the fluorescence data were normalised (see *Materials and methods*) and the fluorescence intensities and paracetamol concentrations were then plotted as functions of time to allow comparison of the two datasets.
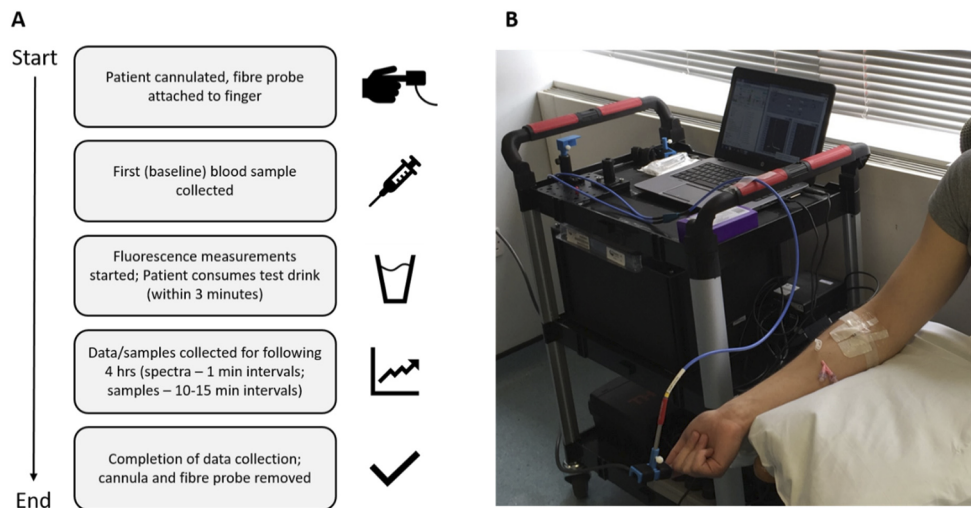

Fig. 2. Experimental methods. **(A)** Flowchart explaining the key steps in the experimental procedure. **(B)** Photograph of a volunteer participating in the experiments. The fibre-optic fluorescence spectrometer (including a laptop for device control) is housed on a wheeled trolley for simple deployment in clinical settings. The fibre-optic probe is attached to the participant's index finger (using a 3D-printed mount) for non-invasive collection of fluorescence data. The participant is also cannulated (below the elbow) to allow collection of the blood samples required for the paracetamol absorption test.

After ingestion of the liquid test meal, both the paracetamol concentration and fluorescence intensity increased over time, reaching peak values within approximately 100 minutes for the majority of participants (Figure S1, Supplement 1). The trends observed in the mean paracetamol concentration and fluorescence intensity exhibited qualitative agreement (Fig. 3(A)), indicating the potential of transcutaneous fluorescence spectroscopy to provide a readout of GER. Interestingly, the relative variability of the fluorescence and paracetamol data were also comparable (Fig. 3(A)), which suggests that the fluorescence data is equally robust to inter-patient variation as the paracetamol concentration data is.

When investigating the individual fluorescence and paracetamol curves as functions of time, it became apparent that two peaks were often present in the fluorescence data while only one peak
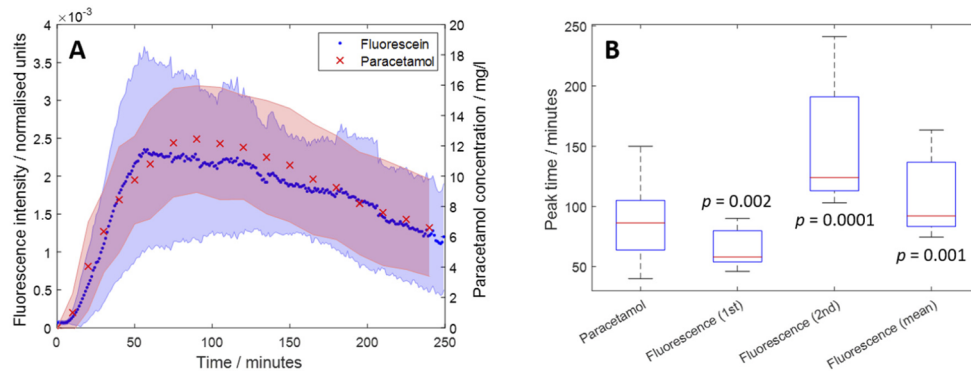
Fig. 3. Comparison of gastric emptying data collected via transcutaneous fluorescence spectroscopy and paracetamol absorption tests. **(A)** Mean fluorescence intensity (blue dots, left axis) and mean paracetamol concentration (red crosses, right axis) as functions of time. Liquid test meal was fully consumed within 3 minutes of the beginning of the experiments in all cases. Values represent means calculated across all participants. Shaded areas represent 1 standard deviation from the mean. Data for one participant (participant 9) was excluded from the calculation of the mean fluorescence intensity due to an error in the fluorescence data collection. **(B)** Box plot showing the times at which peaks were observed in the fluorescence and paracetamol data. As above, data for participant 9 was excluded from the fluorescence boxes. Only one peak was observed in the paracetamol data, hence only one paracetamol box is shown on the plot. Two peaks were typically observed in the fluorescence data. Thus, three fluorescence boxes are shown on the plot: (i) the time of the first peak; (ii) the time of the second peak; and (iii) the mean of the two fluorescence peak times. For each box, the red line represents the median value, the upper and lower edges of the blue box represent the 25th and 75th percentiles, and the whiskers extend to the most extreme data points. P-values represent the results of Wilcoxon signed rank tests comparing the various fluorescence peak times against the paracetamol peak times (for which the data for participant 9 was excluded from all datasets to allow a paired analysis). Statistically significant differences were observed in each case.

occurred in the paracetamol data (see Figures S1&2, Supplement 1). This double peak effect is discussed in [20] and is tentatively attributed to leakage of fluorescein from blood vessels into the epithelium of the skin (which has also been reported elsewhere in the literature, e.g. [29]). This produces a second peak in the fluorescence intensity vs. time curve after the initial peak (which is caused by uptake of the dye from the gut into the blood stream). To further assess the potential of fluorescence spectroscopy for GER assessment we compared the times of the fluorescence and paracetamol peaks. Peak times were identified manually via visual inspection of the fluorescence or paracetamol vs. time curves (Figure S2, Supplement 1). Paracetamol peak times were then compared against both the first and second fluorescence peak times as well as against the average (mean) of the two fluorescence peak times (see Fig. 3(B) and Figure S3, Supplement 1). Interestingly, all three fluorescence peak times were significantly different to the paracetamol peak times, as determined by Wilcoxon signed rank tests (Fig. 3(B)).

3.2. Calculation of percentage retention via fitting of fluorescence vs. time curves

To demonstrate the clinical utility of fluorescence spectroscopy for GER assessment, we calculated the percentage of the liquid test meal remaining in the stomach as a function of time using both the paracetamol and fluorescence data. This 'percentage retention' data is what is typically used clinically when assessing GER. For the paracetamol data, calculation of percentage retention was achieved according to the method presented by Medhus *et al.* [19]. However, because of

the second peak in the fluorescence data and because the relative fluorescence intensities of fluorescein in the blood stream and fluorescein in the skin epithelium were unknown, alternative methods were required for calculation of percentage retention from the fluorescence data. We devised two analysis protocols for this purpose.

In the first approach, the function shown in Eq. (5) was fitted to the fluorescence vs. time data (see *Materials and methods*). Fitting was performed for each acquired fluorescence vs. time curve using software written in MATLAB (MathWorks) incorporating least squares curve fitting. Good fits were obtained in all cases (see Figure S4 and Table S1, Supplement 1), with R^2 values of above 0.85 for all datasets.

Following fitting, we defined the amount of fluorescein that had emptied from the stomach, S, as

$$S(t) = B(t) + B(t)Ct \tag{6}$$

where the term $B(t)Ct$ represents the elimination of fluorescein from the blood stream (which occurs at a rate determined by C). The percentage retention within the stomach, R, was then calculated according to

$$R = 100 \times \left(1 - \frac{S(t)}{S(t_{peak2})}\right). \tag{7}$$

In Eq. (7), t_{peak2} represents the time of the second peak in the fluorescence vs. time curve. This time point was chosen for the calculation of the percentage retention as it represented the point at which no more fluorescein was emptying from the stomach. After t_{peak2}, the fluorescence signals observed for all participants exhibited steady decreases over time (with the rates of decrease varying across participants), indicating that a phase of steady state elimination had been reached in which the only process occurring was elimination of fluorescein from the body.

Using this approach, we compared the percentage retention curves to those calculated from the paracetamol concentration data (see Fig. 4(A) and Figure S5, Supplement 1). Although some deviation was observed at early time points, the mean paracetamol and fitted fluorescence retention curves were in qualitative agreement across the duration of the experiments (Fig. 4(A)). To further investigate the agreement between the paracetamol and fluorescence retention data, we compared the times at which the percentage retention dropped to 75%, 50% and 25% (Figs. 4(B)-(D)). While there was a statistically significant difference between the times at which 75% retention was observed for the paracetamol and fitted fluorescence data (Fig. 4(B)), no significant differences were observed at the time points for 50% and 25% retention (Figs. 4(C)&D). This indicates good agreement between the datasets and suggests that the percentage retention values calculated based on the fluorescence data would be suitable for use as clinical readouts of GER. Importantly, these values were obtained in a non-invasive manner without the need to collect blood (or other) samples. In addition, analysis can be performed directly on the collected data and does not require transport of samples to a laboratory.

3.3. Direct calculation of percentage retention facilitates rapid assessment of GER

While the fitting-based approach described above exhibited good agreement with the paracetamol data, it still required collection of data over several hours (to ensure that good fits were obtained). Thus, we also investigated an alternative, direct method for calculation of percentage retention from the fluorescence data. In this case, the percentage retention was calculated according to

$$R_{Direct} = 100 \times \left(1 - \frac{I(t)}{I(t_{peak1})}\right). \tag{8}$$

Here, $I(t)$ represents the fluorescence intensity data (as a function of time, t) and t_{peak1} represents the time of the first peak in the fluorescence vs. time curve. The time of the first peak was chosen for use in this equation as it represented a point at which most (although not all) fluorescein was
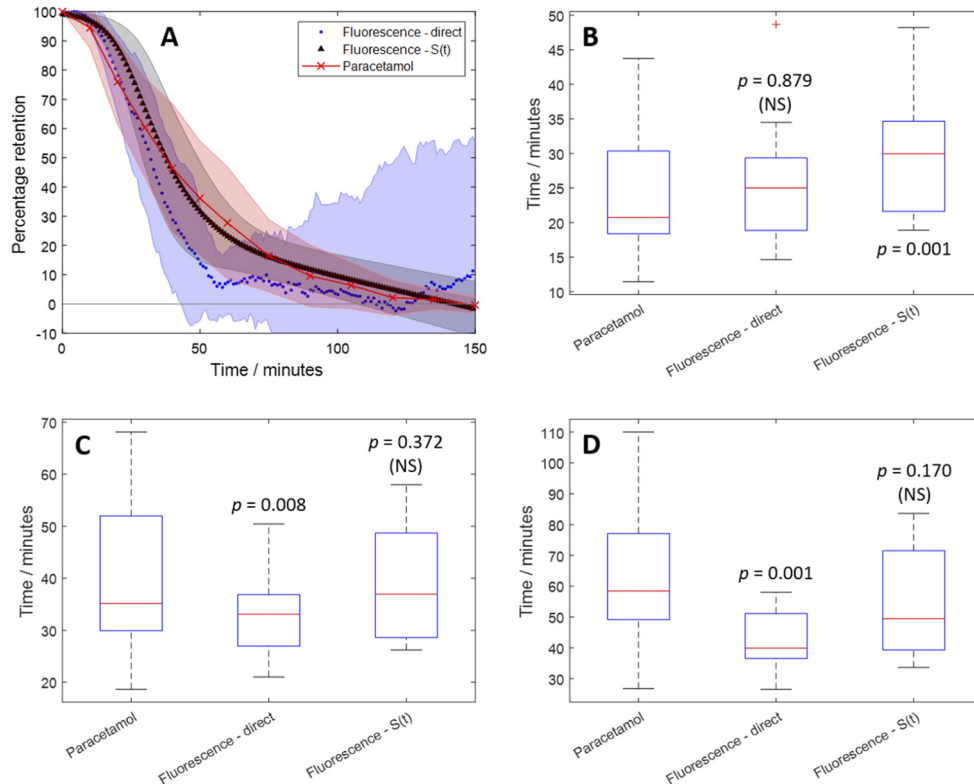
Fig. 4. Comparison of percentage retention (the percentage of the liquid test meal retained within the stomach) calculated based on the fluorescence data (using two methods of data analysis) and the paracetamol data. **(A)** Graph showing percentage retention values as functions of time. Blue dots ("Fluorescence – direct") represent retention values calculated using the direct method of data analysis (see Eq. (8)). Black triangles ("Fluorescence – S(t)") represent retention values calculated via fitting of the fluorescence vs. time curves (see equations (2-7)). Data for participant 9 was excluded from the fluorescence retention calculations due an error in the fluorescence data collection. Data for participant 7 was excluded from the paracetamol retention calculations, as a steady state elimination region was not observed in this dataset (which is required for accurate calculation of percentage retention based on the paracetamol data [19]). **(B-D)** Box plots showing the times at which 75% **(B)**, 50% **(C)** and 25% **(D)** retention were observed using each technique. For each box, the red line represents the median value, the upper and lower edges the blue box represent the 25th and 75th percentiles, the whiskers extend to the most extreme data points (excluding outliers), and outliers are indicated by red '+' symbols. As above, data for participants 7 and 9 were excluded from the paracetamol and fluorescence boxes respectively. P-values represent the results of Wilcoxon signed rank tests comparing the fluorescence retention times against the corresponding paracetamol retention times (for which the values for participants 7 and 9 were excluded from all datasets to allow a paired analysis).

likely to have emptied from the stomach and also allowed calculations to be performed using data collected over the first 100 minutes (or less) after ingestion of the liquid test meal. Hence, this approach could permit faster clinical assessment of GER.

Using this method, clear differences were apparent in the percentage retention curves (compared to the paracetamol data) at later time points (Fig. 4(A)). However, good agreement was observed over the early time points, up to approximately 60% retention (Fig. 4(A); as shown on the figure, the direct method agrees well with the paracetamol data at time points up until 60% retention, while the fitting-based approach provides better agreement at times beyond 60% retention). Furthermore, when comparing the time points for 75%, 50% and 25% retention (Figs. 4(B)-(D)), we observed a significant difference between the paracetamol values and the directly calculated fluorescence values at 50% and 25% retention. At 75% retention, however, no significant difference was observed.

To further analyse the agreement between the percentage retention values from the paracetamol and fluorescence data, we plotted Bland-Altman graphs (Fig. 5). These display the difference between the paracetamol and fluorescence retention times (for both the fitting and direct calculations) against the average of the paracetamol and fluorescence retention times. For the direct method for calculation of retention from the fluorescence data, the times at which 75% retention is reached are in excellent agreement with the paracetamol values (Fig. 5(A)). In addition, no trend is observable in the time differences as a function of average time (Fig. 5(A)). For the 50% and 25% retention times, however, larger average differences are observed and clear trends are present in the data, with higher average retention times correlating with larger time differences (Figs. 5(B)&C). For the fitting-based analysis, on the other hand, trends are much less obvious for all three retention times (Figs. 5(D)-(F)). Furthermore, the differences between the paracetamol and fluorescence retention times are small for all time points, and the agreement in the data is particularly strong for the 50% and 25% retention times (when considered as proportions of the average retention times) (Figs. 5(D)-(F)).
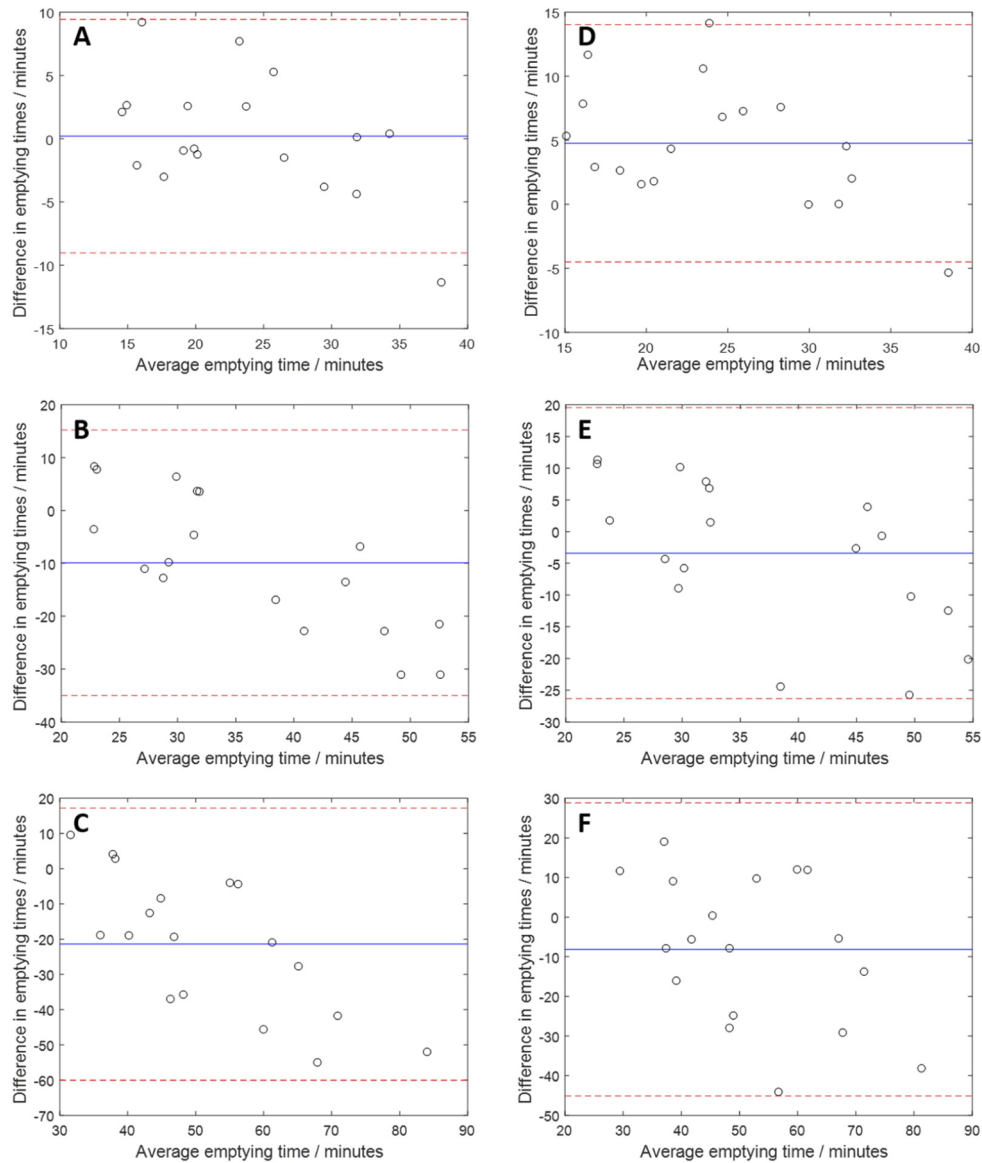
Fig. 5. Bland-Altman plots comparing the emptying (retention) times measured using transcutaneous fluorescence spectroscopy against paracetamol absorption tests. Graphs show the difference between the emptying (retention) times (i.e. fluorescence emptying time minus paracetamol emptying time) against the average (mean) of the fluorescence and paracetamol emptying (retention) times. **(A-C)** Bland-Altman plots for the direct method of calculation of percentage retention from the fluorescence data (see Eq. (8)) for retention values of 75% **(A)**, 50% **(B)** and 25% **(C)**. **(D-F)** Bland-Altman plots for the fitting-based method of calculation of percentage retention from the fluorescence data (see equations (2-7)) for retention values of 75% **(D)**, 50% **(E)** and 25% **(F)**. Solid blue lines indicate the mean difference in emptying (retention) time. Dotted red lines represent the 95% limits of agreement (i.e. mean ± 1.96 standard deviations). For all plots, data for participants 7 and 9 were excluded from the analysis due to the paracetamol data not reaching steady state elimination (participant 7) and an error in the fluorescence data collection (participant 9).

4.   Discussion

The results reported above demonstrate the use of a new technique – transcutaneous fluorescence spectroscopy – for non-invasive assessment of GER. Using this method, participants consume a liquid test meal containing a fluorescent contrast agent (in this case, fluorescein) and a wearable probe detects the fluorescence signal in the blood stream as the agent passes from the gut into the blood.

To assess whether the resulting fluorescence vs. time curves could be used for clinical measurement of GER, we compared our new technique against the paracetamol absorption test (a clinically approved test for GER) in 20 healthy volunteers. While the raw paracetamol and fluorescence data showed qualitative agreement, differences were observed, in particular in the times at which peak values occurred.

Thus, we devised two methods to calculate percentage retention from the fluorescence data and compared the resulting values against paracetamol retention data (which was calculated according to the method presented in Ref. [19]). The fitting-based approach (equations (2-7)) provided values that were in good agreement with the paracetamol retention data, particularly at longer time points (i.e. 50% and 25% retention). Where the percentage retention values from the fitting-based approach differed from those obtained using the paracetamol absorption test (i.e. at 75% retention), these discrepancies can be attributed to limitations in the model used for fitting and/or to physiological differences in the ways in which paracetamol and fluorescein are processed in the body. For example, paracetamol is primarily eliminated from the body by the liver while fluorescein is eliminated by both the liver and the kidneys (with approximate rates of clearance of fluorescein reported as 1.75 ml/min/kg and 1.5 ml/min/kg for the renal and hepatic pathways respectively [30]). Despite this, we chose to fit a single rate of clearance for fluorescein in our model. We took this approach in order to keep the model as simple as possible (thereby avoiding problems caused by overfitting) and because the data indicated that it provided good fits and therefore good estimates of the total clearance that had occurred at any given time point (i.e. a single rate of clearance appeared to provide a good approximation of the overall clearance process). As such, it was sufficient to provide accurate calculation of GER in this dataset. Nonetheless, in future work, we intend to explore alternative and improved models to provide more accurate assessment of percentage retention at all time points. Importantly though, it is noteworthy here that even existing, clinically approved methods for assessment of GER exhibit differences in the percentage retention values they return (e.g. see [15]). Thus, the results obtained with our fitting-based technique already imply potential for accurate clinical assessment of GER.

The direct method of calculation (Eq. (8)), on the other hand, was less accurate at later time points (i.e. for 50% and 25% retention) but showed excellent agreement with the paracetamol data at earlier times (75% retention). Importantly, the time points for 75% and 25% paracetamol retention were correlated with one another (i.e. longer 75% retention times were predictive of longer 25% retention times – see Figure S6, Supplement 1). Hence, while the direct method sacrificed accuracy at longer time points, our data nonetheless demonstrates that it has potential for rapid clinical assessment of GER (i.e. based on data collected within 30–100 minutes of liquid test meal consumption).

Importantly, the fluorescence-based approach presented here is less invasive than current methods for assessment of GER, does not require collection of blood (or other) samples, and does not require nuclear medicine facilities (which are necessary for gastric scintigraphy). In addition, both analysis methods allow for immediate interpretation of data without the need to send samples to a laboratory. Thus, both can facilitate faster reporting of clinical results than current techniques.

The direct analysis method will allow for data collection (and analysis) within a total test time of as little as 30 minutes. However, as data can potentially be analysed immediately using the

same computer that is used to control the data collection, even the slower fitting-based approach will allow for reporting of clinical results within approximately 4 hours – i.e. the results can be reported as soon as the test is finished as no laboratory analysis is required. Thus, transcutaneous fluorescence spectroscopy has numerous clear advantages over the current standard of care: it is non-invasive, no sample collection or laboratory analysis is required, it is fast, and it has the potential to reduce costs.

Finally, the method presented here used fluorescein as a contrast agent, which is already clinically approved for many other clinical procedures (e.g. ophthalmic angiography). Combined with the numerous benefits described above, this implies the potential for rapid clinical uptake of this new approach for quantification of GER. In turn, this would provide opportunities for improved monitoring of a wide range of clinical conditions. This could include, for example, diagnosis of gastroparesis, monitoring of recovery from bariatric surgery, and even screening for patients at high risk of stomach cancer.

Nonetheless, there are of course areas where this technique could be improved. First, as discussed above, improved models for the fitting analysis could facilitate more accurate assessment of GER. Second, using a fibre probe that allows deeper penetration into tissue (e.g. through use of larger spacings between the source and detector fibres) may provide enhanced sensitivity to the dye within the blood stream (rather than the dye that has leaked out of blood vessels towards the surface of the skin). This may reduce the double peak effect observed in the data presented here and, hence, could act to make quantification of GER simpler. Third, a lower cost and more portable device would also be beneficial. Interestingly, it may be possible to collect similar data to that reported here using a simpler, smaller and cheaper device. For example, utilizing light emitting diodes for excitation and single channel detectors (e.g. photodiodes) in combination with suitable band pass filters for detection (rather than a laser and a spectrometer respectively) could potentially facilitate significant reductions in both size and cost. Our ongoing research will focus on these points, with the key aim of developing systems suitable for wider clinical testing in the near future.

5. Conclusions

In conclusion, transcutaneous fluorescence spectroscopy of orally ingested fluorescein allows rapid, non-invasive assessment of GER. We observed good agreement between this new technique and paracetamol absorption tests, which are clinically approved for assessment of GER. Crucially, transcutaneous spectroscopy is non-invasive and does not require sample collection or laboratory analysis. Furthermore, we reported two techniques for data analysis, both of which will facilitate rapid reporting of clinical results in hours rather than days. Overall, this indicates clear potential for improved monitoring and assessment of numerous clinical conditions in which GER is altered, and the advantages over the current standard of care imply the potential to facilitate clinical uptake of such tests on a much larger scale.

6. Author Contributions

A.J.T. conceived the project and developed the fibre-optic spectrometer. A.J.T., A.M.L., J.M., N.V., A.D., R.G. and M.T. designed the clinical experiments. A.J.T, A.M.L., J.M., R.G. and M.T. prepared documents for the ethics application. A.J.T., A.M.L., A.L. and C.S. performed the experiments. A.J.T. and A.M.L. analysed the paracetamol data. A.J.T. analysed the fluorescence data. A.J.T. and A.M.L. wrote the manuscript. All authors edited and approved the manuscript.

Disclosures. A.J.T. is inventor of a patent relevant to the use of transcutaneous fluorescence spectroscopy as a tool for non-invasive monitoring of gastric emptying rate and the potential recipient of royalty payments related to this method. All other authors declare no competing interests.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

Supplemental document. See Supplement 1 for supporting content.

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